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BIOLOGICAL BULLETIN

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.

VI. Double- and Triple-Yolked Eggs.[1]

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The most common type of abnormal egg produced by the domestic fowl is the double-yolked egg. Eggs with three yolks, however, are very rare. The purpose of the present paper is, first, to record some observations on the frequency of the occurrence of double- and triple-yolked eggs and the relation of their production to the age of the bird; and, second, to consider so far as possible the nature of the processes involved in their formation and the contribution that such a study makes to our knowledge of the physiology of egg production.

The Frequency of Occurrence of Double- and Triple-Yolked Eggs and the Relation of Their Production to the Age of the Bird.

During the last six years only three triple-yolked eggs are known to have been produced by the flock of birds belonging to

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 56.

The previous papers in the series of "Studies on the Physiology of Reproduction in the Domestic Fowl" are:

I. Pearl, R., "Regulation in the Morphogenetic Activity of the Oviduct," *Jour. Exp. Zool.*, Vol. 6, pp. 339–359, 1909.

II. Pearl, R., and Surface, F. M., "Data on the Inheritance of Fecundity Obtained from the Records of Egg Production of the Daughters of 200-egg Hens," Me. Agr. Exp. Sta. Ann. Rept., 1909, pp. 49–84.

III. Pearl, R., and Curtis, M. R., "A Case of Incomplete Hermaphroditism," Biol. Bull., Vol. 17, pp. 271–286, 1909.

IV. Pearl, R., and Surface, F. M., "Data on Certain Factors Influencing the Fertility and Hatching of Eggs," Me. Agr. Exp. Sta. Ann. Rept., 1909, pp. 105–164.

V. Pearl, R., and Curtis, M. R., "Data Regarding the Physiology of the Oviduct," *Jour. Exp. Zool.*, Vol. 12, pp. 99–132.

the Maine Agricultural Experiment Station. The poultrymen have been under instructions to bring all abnormal eggs to the laboratory where they are opened. It seems hardly probable that they would fail to observe the abnormal size of a triple-yolked egg. During the six years more than three thousand different adult birds have been kept, each for one or more years. If no triple-yolked eggs have escaped notice, fewer that one bird in a thousand have laid triple-yolked eggs.

Each of the three triple-yolked eggs produced in these flocks was laid by a different bird. In each case the triple-yolked egg was one of the first six eggs produced by a pullet which began to lay when between five and six months old. This indicates that young pullets are more likely to produce this type of abnormal egg than are older birds. Fowls show also a greater tendency to lay double-yolked eggs at this age than when more mature. At this stage of development the time of successive ovulations is less precisely regulated than later in life. It should be kept in mind, however, that even at this age there are many more birds which do not lay multiple-yolked eggs than there are which do, and that usually one bird lays only one such egg.

Data collected during two investigations in the physiology of egg production show incidentally what percentage of the pullets which began to lay when between five and seven months of age produce eggs with more than one yolk. The first of these observations was made in the fall of 1910. At that time data were taken on all the eggs laid by a small flock of pullets. Twenty of these pullets began to lay when under seven months of age. Among the first ten eggs of four (or 20 per cent.) of these there was one or more which was double-yolked. The second observation was made in the fall of 1913 when one hundred and sixty-nine pullets laid before November 1. All of these were less than seven months old. Thirty-three of them or 19.5 per cent. laid one or more eggs with more than one yolk. The data on the laying of multiple-yolked eggs by these two flocks of pullets are brought together in Table I.

This table shows that in 80 per cent. of the birds of this age the normal rhythm of ovulation, and the successive stages of egg formation which result in the enclosing of each yolk in

TABLE I.

SHOWING THE NUMBER OF MULTIPLE-YOLKED EGGS PRODUCED BY PULLETS FIVE TO SEVEN MONTHS OLD.

No. of Eggs with More than one Yolk.	No. of Pullets Observed.	Percentage of Observed Flock.
0	152	80.42
1	26	13.76
2	8	4.23
3	3	1.59

separate envelopes, are sufficiently well regulated at the beginning of laying to result in the production of only normal single-yolked eggs. The other 20 per cent. of the birds produce one to three eggs with more than one yolk. Less than 6 per cent. of the flock, however, show more than one such irregularity while only about 1.5 per cent. show three such imperfections of regulation. It is thus clear that even in young birds ovulation and egg formation is in general a well-regulated process. Disturbances in the rhythm are, however, most likely to occur among young birds.

The question naturally arises as to whether it is the immaturity of the bird or the lack of laying experience which is responsible for the unstable regulation of ovulation. If birds which begin to lay younger show a more decided tendency to produce eggs with more than one yolk while those which are more mature produce only normal single-yolked eggs, it might be concluded that immaturity is associated with a lack of precision in the regulation of the time of successive ovulations.

Data now being collected at this laboratory will probably be sufficient to answer this question. The evidence at present available, although insufficient to settle the question, suggests that immature birds produce more multiple-yolked eggs than mature birds which have not laid when young. The evidence consists of the three following observations. First, only about one-third of the pullets of all sorts, including many crosses not carrying hereditary factors for high winter production, which are kept at the station, begin to lay before November 1. The others begin to lay at varying times up to March. A few double-yolked eggs are produced in every month of the year, but by far the greatest number are produced at the end of September and

during October when the pullets which are coming on to lay are immature. This observation does not agree with the statements of Immerman,[1] Panum[2] and Parker[3] that the greatest number of double-yolked eggs occur in the warmer part of the year. Immerman, however, recognized the fact that the seasons when he obtained the largest number of double-yolked eggs coincided with the periods of highest egg production. He believed that the laying of double-yolked eggs occurred during the entire laying period and that no season had an especial influence on their production. Since the eggs on which these workers base their statements were collected from the market, or brought to them by their friends, it is likely that they did not receive the double-yolked pullet eggs, which are often not distinguishable externally from the single-yolked eggs of the year-old and two-year-old hens.

It is also possible that the birds in the part of the country where these people worked were so bred and handled that they did not begin to lay until much later than birds specially bred for winter production.

The second observation which suggests that an immature bird is more likely to produce double-yolked eggs than one which is mature was made upon the eggs of a single pullet which began to lay when she was exactly three months old and before she had assumed her adult plumage. The first ten eggs of this pullet were kindly presented to this station by the owner of the bird, Mr. Walter Gerald, of Unity, Maine. All of these ten eggs were smaller than a normal pullet's egg but the four largest of them contained two yolks. This pullet, which began to lay when much less mature than any of the pullets which have been raised on the station plant, produced more double-yolked eggs than have been laid by any of these birds. This bird may, of course, be absolutely abnormal in respect to the production of double-yolked eggs.

[1] Immerman, F., "Über Doppeleier beim Huhn," Inaugural-Dissertation, Basel, 43 pp., 1899.

[2] Panum, P. L., "Untersuchungen über die Entstehung der Missbildungen zunächst in den Eiern der Vögel," Berlin, 260 pp., 1860.

[3] Parker, G. H., "Double Hen's Eggs," *American Naturalist*, Vol. XL., pp. 13–25, 1906.

It might also be repeated here that the three triple-yolked eggs were all produced by pullets less than six months old.

The third observation is that only normal single-yolked eggs were produced by three pullets which did not lay until they were 8¾ to 9 months of age. Every egg of these birds was opened. Since 80 per cent. of the pullets between 5 and 7 months of age lay only normal single-yolked eggs, this third observation has no statistical value, but it is given since it is the only absolutely accurate data at present available on the eggs of the birds which begin to lay when fully mature.

The kind of disturbances which result in the formation of double- and triple-yolked eggs are not yet thoroughly understood. From the preceding paragraphs it would appear that whatever their nature they are more apt to occur in immature than in mature birds. Yet during six years observation, the Experiment Station's flock, consisting each year of from 600 to 800 birds, has produced double-yolked eggs during every month of the year. This accords with Immerman's observations in Basel and indicates that such disturbances may occur at any time.

Parker quotes Landois'[1] statement that the laying of double-yolked eggs may become habitual with certain hens and also cites the single cases of such birds noted by Bartels[2] and Immerman.[3] A third single case has since been reported by Glaser.[4]

Not a single case of this kind has occurred among the large number of birds owned by this station during the last six years. This fact together with the small number of the actual cases cited in the literature indicates that birds which habitually lay double-yolked eggs are very rare. The occasional occurrence of such cases, however, indicates that physiological disturbances capable of causing such a result may become chronic.

Data from the station flock, however, indicate that even when the disturbances are not chronic they may recur several times in one individual, *i. e.*, an individual may show a predisposition

[1] Landois, "Missbildungen bei Hünner-Eiern," *Zool. Garten*, Jahrg. 19, pp. 17–24.

[2] Bartels, M., "Hühner Ei mit Zwei Dottern," Sitzungs-Ber. Gesellsch. naturf Freunde Berlin, Jahrg. 1895, pp. 143–145.

[3] Immerman, *loc. cit.*

[4] Glaser, O., "The Origin of Double-Yolked Eggs," BIOL. BULL., Vol. XXIV., pp. 175–186.

to them. Table I. showed that some pullets before they were seven months old had produced as many as three eggs with more than one yolk, while 80 per cent. of the flock had laid only single-yolked eggs. Also the data collected for the investigation on egg size contribute some evidence on this point. All of the eggs laid by 22 birds during their first two laying years were opened. Only 5 of these birds ever laid an egg which had more than one yolk. Four of these five laid one or two double-yolked eggs among their first seven. Two of the four never afterward laid an abnormal egg. The others, however, produced two (in the first case) or three (in the second) double-yolked eggs when they were mature. Out of the flock of 22 only one bird which had not produced double-yolked eggs at the beginning of the first laying period produced one after she was mature. This indicates that individuals differ in the stability of the regulation of their reproductive processes, and if these processes in an individual are unstable, irregularities are most likely to occur among the first eggs produced when the bird begins to lay while young. Also different individuals show varying degrees of instability, so that some may also show irregularities later.

The Relation of the Production of Double-Yolked Eggs to Other Forms of Abnormally Rapid Egg Production.

A study of the nature of the disturbances which cause the formation of double- and triple-yolked eggs is limited by the fact that as yet we do not fully understand the nature of the regulation of the egg-forming processes which cause the production of successive single-yolked eggs. One of the earliest and most extensive investigations of the physiological processes involved in egg formation was that of Coste.[1] He made important observations on the time spent by the egg in the oviduct. Similar observations are also recorded in a previous paper from this laboratory.[2] Published and unpublished observations show that

[1] Coste, M., "Histoire du développement des corps organisés," Tome I., Paris, 1874.

[2] Pearl, R., and Curtis, M. R., "Studies in the Physiology of Reproduction in the Domestic Fowl, V., Data Regarding the Physiology of the Oviduct." Jour. Exp. Zool. Vol. 12, pp. 99–132.

normally a yolk is ovulated into the mouth of the oviduct and passes down the duct, receiving the chalazal layer,' chalazæ, albumen, shell membrane and shell. The egg remains in the oviduct several hours after the shell is formed. In the case of a few birds which were laying every day a second yolk has been found in the upper part of the oviduct while there was still a hard shelled egg in the shell gland. These birds were killed after they had gone on the nest but before they had laid. That is, when birds are laying at daily intervals two eggs may be normally present in the duct at the same time. In order that two eggs may be separate single-yolked eggs it is only essential that the second does not overtake the first. If the two eggs keep moving toward the cloaca the time between successive ovulations may be somewhat reduced without causing any form of doubling of the egg. What happens is a reduction in the time between the laying of the eggs. Ordinarily the period between two eggs of a clutch is somewhat more than 24 hours. However, some of the best laying birds at the height of their reproductive period lay in less than 24-hour periods. For several days they may lay somewhat earlier each day than on the preceding day.

One egg every day is the maximum normal egg production. Occasional cases have been reported where two eggs were produced at different times during the same day. Most such cases do not bear critical investigation. For example, at this and other poultry plants where trap-nests are used, a bird which is already credited with an egg on that day is sometimes found apparently to have laid again. The probability that the bird has actually laid two eggs is much smaller than the probability that the poultryman misread the first band number or some other slip occurred. Unless there is some independent check the evidence cannot be accepted. Such a check was possible in several such cases at this plant. One may be mentioned here. The eggs of bird No. 771D were being saved for incubation and each was marked with the bird number when it was taken from the trap-nest. Therefore, when the second egg was taken from the nest on May 19, 1908, the first egg had not been mixed with the eggs of other birds. The resemblance between the two eggs was sufficient to make it certain that the same bird had produced

them. This bird had laid on each of the three preceding days, but did not lay on either of the two following days. The production of the two eggs in one day in this case may have been due to a shortening of the time between the last two ovulations in the clutch although it is also possible that it was due to the early expulsion of the last egg.

An authentic record of a White Wyandotte pullet which showed a rate of production high enough to cause several instances of the production of two eggs in a day has been published by Drew.[1]

Other cases have been recorded where two separate eggs have been produced at the same time. As in the preceding case all such records must be most critically investigated since an egg may have been overlooked the last time the nest was used or a bird may have entered a nest, laid and gone out while another bird was standing on the open trap door. A second bird may later be found in the nest with two eggs. Therefore, no record of two eggs at one time is accepted at this plant unless the two eggs so closely resemble each other and differ so decidedly from the average eggs of the breed that there can be no doubt that they were produced by the same bird. Four such undoubted cases of the laying of two separate normal eggs at one time have occurred at this plant. Two of the birds producing these had laid on the preceding day but did not lay on the following. These cases then must be accounted for by the unusually rapid succession of the second egg. One of the birds produced an egg on both the preceding and following days. In this case also the only possible explanation is an abnormally rapid period of egg production. In the fourth case the bird produced an egg on the following but not on the preceding day. This case may represent abnormally rapid production, or it may simply be that the first egg failed to be discharged from the duct until the second one was completely formed. These cases all show that an egg may overtake its predecessor after they are both completely formed. In some cases this is most probably due to a decrease in the normal minimum time between ovulations, although the same effect (*i. e.*, production of two eggs at the same time) is

[1] Drew, Gilman A., "Hens that have Laid Two Eggs in a Day," *Science*, Vol. 26, N.S., pp. 119–120, 1907.

produced by the holding of the first egg in the duct after it would normally be laid.

A few cases of egg-bound birds have come to autopsy in which the uterus contained a second fully mature egg. These cases do not of course necessarily indicate an unusually rapid egg production. One case, however, came to autopsy where there were two normal thin shelled eggs in the uterus. Neither one seemed to have the shell completely formed. There was no evidence that the eggs could not be laid. This appeared to be a case where two eggs were being completed at the same time. The bird had never laid.

These cases have been described because it seems clear that the kind of disturbances which cause the production of eggs with more than one yolk may also, when slighter in extent or when localized in a more posterior portion of the oviduct, cause other irregularities in egg production.

There is in fact a certain type of abnormal egg which forms the logical step between two eggs at one time and the double-yolked egg. Such eggs, although occurring very infrequently, have several times been described in poultry journals. One of them has been produced at this plant. These abnormalities consist of pairs of normal eggs united by a tube of membrane containing albumen. In the pair of such eggs produced at this plant the components were both soft shelled, one of them having a little more shell than the other. In such cases the second egg must have so nearly approached the first that the stimuli upon the isthmus glands overlapped, causing the formation of the membranous tube continuous with the shell membrane of each. It is interesting to note that the bird which produced this egg had produced a double-yolked egg six days before. Mr. F. E. Field,[1] of Birmingham, England, who described and published a photograph of such an egg, also notes that the pullet which produced it "has since laid several double-yolked eggs." The relation between such eggs and certain types of double-yolked eggs is obvious. If the second egg actually overtakes the first before the first has completely entered the isthmus a double-

[1] Field, F. E., "Peculiar Twin Eggs," *Poultry World* (London), Vol. II., N.S., pp. 1152, 1913.

yolked egg will result. The relation of the two yolks to each other and to the surrounding egg envelopes will depend upon the particular part in the duct above the isthmus ring where the two eggs join, and upon the nature of the processes which bring the two eggs together.

The Nature of the Disturbances in Normal Egg Production which Bring two Yolks together in the Oviduct, and the Influence of the Part of the Duct where they Join upon the Relation of the Yolks to Their Enclosing Envelopes.

Parker,[1] in his clear and logical discussion of double hens eggs, explains the cause of double-yolked eggs as the simultaneous discharge of two yolks either from the same or separate ovarian follicles. This is the most simple explanation of the class of double-yolked eggs where the two yolks have apparently passed the entire length of the duct together, as evidenced by the enclosure of the two yolks in a single set of envelopes, beginning with the chalaziferous layer. In such cases the two yolks are often very much flattened together and held by a thin, firm chalaziferous membrane. These yolks have a single set of chalazæ and are enclosed in a single envelope of thick albumen. A large number of eggs of this type are produced by the station flock every year. In this group would fall eggs belonging to both of the sub-classes of double-yolked eggs described by Immerman,[2] that is, those in which the yolks are enclosed in a single vitelline membrane, and also those in which the yolks have separate vitelline membranes. In fact all of the eggs of the first class must fall in this group. The occurrence of two yolks within the same vitelline membrane is, however, very rare among the eggs laid by the station flock. There has indeed been but one such case. In the other apparent cases it was possible by very careful dissection to remove the chalaziferous layer or chalazal membrane and separate the two yolks.

While Parker's explanation is sufficient to account for this group of double-yolked eggs it does not account for double-

[1] *Loc. cit.*

[2] *Loc. cit.*

yolked eggs where the two yolks have some separate envelopes. Nor indeed is it the only possible explanation for double-yolked eggs where all the envelopes are common to the two yolks.

Glaser[1] suggests that a failure of the peristalsis of the duct just below the infundibulum, due either to "a deficiency of substance normally inducing the movements or (to) the subnormal irritability on the part of the oviduct," might result in a yolk remaining in the upper part of the duct until a second was normally ovulated. The two might then pass down the duct together. Whether or not this ever happens is not known, but it is, certainly, theoretically possible.

Glaser's second suggestion as to an additional cause for the formation of double-yolked eggs is based upon the case he describes where the laying of double-yolked eggs was "habitual." The suggestion is that in such cases the laying of double-yolked eggs may have a "distinct ovarian basis" which in this case he believes to be the tendency to a secondary fusion of follicles which results in a common blood supply. This seems rather to be a suggestion of the nature of the underlying cause of the synchronous maturity and discharge of two or more yolks. The fusion of follicles and a common blood supply may be an important cause for simultaneous ovulations.

There is, however, one additional possibility for the formation of double-yolked eggs with a single set of egg envelopes. A yolk may be ovulated into the body cavity and remain in that portion of it which is walled off into a sort of ovarian pocket[2] formed by the mesentery, cœca, and air sac wall. It is thus near the mouth of the oviduct. If this free yolk entered the infundibulum just before or immediately after a second ovulation the two yolks might pass down the duct together. A free yolk has never been seen entering the oviduct but many observations of ovulation into the body cavity and the anatomical relation of the infundibulum to the ovary make it necessary to list this among the theoretically possible causes for the presence of two yolks in the duct at the same time.

[1] *Loc. cit.*

[2] This pocket has been described by the author in a previous paper. Curtis, M. R., "The Ligaments of the Oviduct of the Domestic Fowl," Ann. Rept. Me. Agr. Exp. Sta., 1910, pp. 1–20.

The two yolks of a double-yolked egg often possess some separate envelopes, indicating that they have not passed through the entire length of the duct together. In fact a careful study of a large number of double-yolked eggs indicates that the second may join the first at any point between the infundibulum and the isthmus ring.

Two separate yolks with a common set of envelopes may be so closely united by the chalaziferous layer that they appear to have also a common vitelline membrane. Such pairs of yolks are very much flattened together. There is a complete and graded series from this condition to one where the two yolks barely touch one another. In the latter case the two yolks have really chalazal membranes which are practically separate although they still have but one set of chalazæ. The two yolks have evidently passed down the duct in quick succession but the first has preceded the second sufficiently to allow each to preserve its form.

This group of double-yolked eggs passes insensibly into the group in which the yolks have separate chalaziferous layers but all the other envelopes in common. Fig. 1 shows diagrammatically the typical relations of the two yolks in double-yolked eggs where the yolks have common albumen envelopes.

Where the arrangements of the two yolks and the chalazæ are those shown in Fig. 1, *c* to *f*, the second yolk must have overtaken the first after the chalazal layer was formed and before the thick albumen was secreted; that is, near the beginning of the albumen secreting portion of the duct. In the cases figured the stimulations given by the two yolks upon the glands which cause the formation of the chalazæ must have overlapped. In cases like Fig. 1, *f* the chalazæ sometimes appear thin in the middle. This may be due to the faintness of stimulation on the glands or to stretching of the chalazæ as the yolks are bent on their axes. A few double-yolked eggs with one set of albumen envelopes have been observed where the two yolks had separate chalazæ and several where one yolk had two and the other had none that were visible. It should be stated that in all types of double-yolked eggs the chalazæ of the second yolk, (*i. e.*, the one nearest air cell of the egg) are often less well developed than those

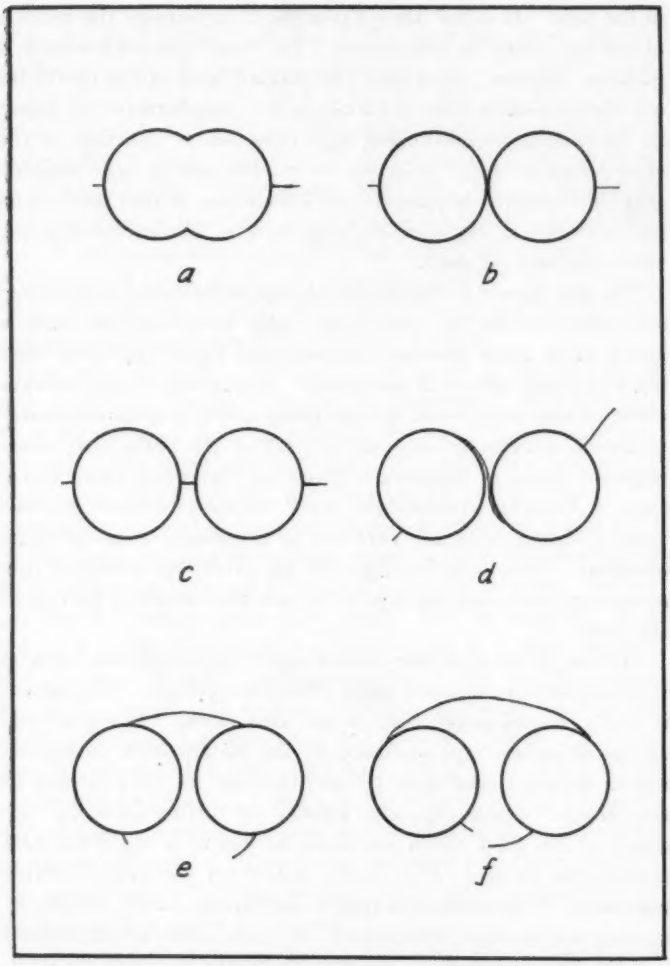


FIG. 1. Diagrams showing the various relations of the two yolks in double-yolked eggs where the yolks have common albumen envelopes. *a*, two yolks of a double-yolked egg which have all the envelopes in common; *b*, the form transitional between a double-yolked egg where the chalazal membrane is common to the two yolks and where each has a separate chalazal membrane; *c*, *d*, *e* and *f*, showing arrangements of the two yolks which have been observed in double-yolked eggs where the yolks have separate chalazal membranes but common albumen envelopes.

of the first. It is not always possible to determine the relation of the two yolks by this means. The many cases where this is possible, however, show that the chalazal axes of the two yolks are related to each other in various ways. Immerman[1] has shown by incubating double-yolked eggs that this is also true of the embryological axes. A study of double-yolked eggs suggests that this variation in position of the axes may in part result from the difference in the time elapsing between the entrance of the two yolks into the duct.

The next group of double-yolked eggs includes the transitional form between the eggs that have yolks with separate chalazæ and a single thick albumen envelope and those with yolks that have separate albumen envelopes. This group is not always easily distinguished from the last group and it is often impossible to decide whether or not one or both of the yolks have some separate layers of albumen. There are, however, cases where there is certainly considerable thick albumen between the two yolks although both are enclosed in a common mass of thick albumen. This type of egg will be produced whenever the second egg overtakes the first in the albumen secreting portion of the duct.

The last group of double-yolked eggs to be considered contains those which have separate thick albumen envelopes. This group is easily distinguished and is not uncommon. Seven of the 44 double-yolked eggs produced by the station flock during the month between September 20 and October 20, 1913, belong to this group. These eggs can usually be distinguished by the shape of the shell which has more or less of a depressed ring around the center. The double nature of the egg is visible externally. The depressed ring is sometimes hardly visible, or again it may be very pronounced. In cases where the depression is slight there is usually only a smooth membrane inside the shell, but in cases where it is more pronounced there is sometimes at the base of the depression a very narrow shelf of membrane which extends all or part of the way around the shell. There can be no doubt that in this group the two eggs join while passing into the isthmus and that the depth of the depression depends upon the time during this passage when the two eggs unite.

[1] *Loc. cit.*

The posterior of the two eggs (that is, the one toward the air cell in the shell membrane) has a less deep but more viscous envelope of thick albumen than the anterior one. In consequence the anterior portion of the shell is larger. The relative position of the chalazal axes of the two yolks cannot always be determined, since, as noted above, the chalazæ of the second yolk are often not distinguishable. The chalazæ of the first yolk are always, so far as observed, in the same position as in a normal single-yolked egg. A few cases have been observed where the chalazal axis of the second egg was also in normal position. In one case, however, where the chalazæ of the second yolk were visible, the chalazal axis was turned about 30° from normal.

This group of eggs is the end of the series of double-yolked eggs, since when an egg overtakes its predecessor after that egg has passed entirely into the isthmus, the result is no longer two eggs within one membrane and shell. The relation of this group to twin eggs connected by a membranous tube is obvious.

In all double-yolked eggs where one or more of the egg envelopes is not common to the two yolks, it seems that instead of the simultaneous entrance of two yolks into the oviduct, the first precedes the second by a short time. This is probably most often due to the ovulation of a second yolk at a short interval after the first. It may sometimes be due to the fact that either the first or second yolk to enter the duct was ovulated into the body cavity and was taken up by the duct shortly before or immediately after the next ovulation. Or perhaps the first egg may sometimes come to a place in the oviduct which is subnormally sensitive to peristaltic stimuli, and remain there until joined by the second yolk. There is also a fourth possibility, namely that antiperistaltic movements may carry the first egg back up the duct until it meets the second.

Parker[1] called attention to the fact that the occurrence of antiperistalsis of the oviduct is proven in the cases where fully formed eggs are found in the body cavity. He further shows that only antiperistalsis can account for the enclosure of an egg within an egg. If an egg which has received its shell membrane, or its shell membrane and shell, is carried back up the duct and

[1] *Loc. cit.*

there meets another, and the two come down together, a double egg (*ovum in ovo*) will be formed. If, however, an egg which has not received its shell membrane is carried back up the duct and meets another coming down the result will be some form of double-yolked egg, the particular sort depending upon the relative development of the two eggs when they unite. The conditions under which an egg without a shell membrane would find another egg in the duct even if returned by antiperistalsis are not usual, since a second yolk does not normally enter the duct until some time after its predecessor has received a shell membrane. Such conditions might be brought about either by the entrance of a second yolk at an abnormally short interval after the first or by the first egg remaining stationary for some time or returning slowly.

It has been shown that the conditions necessary for the formation of a double-yolked egg (or a double egg) are that the two eggs unite in the oviduct and then proceed through the duct together. In all cases where the structure of the egg shows that the two components have not passed the entire length of the duct together the passage of the second egg must have been more rapid than that of the first for should the two eggs continue to move through the duct at the same rate they would only unite in the cases where their entrance was practically or absolutely simultaneous. In the preceding paragraphs the possibility of the delay or return of the first egg has been discussed. One further possibility is that the second yolk may pass through the duct at an abnormally rapid rate.

It is thus possible that double-yolked eggs do not always represent a simultaneous or abnormally rapid succession of two ovulations since a yolk may sometimes remain in the oviduct, or body cavity, until the time normal for a second ovulation. There are some cases of double-yolked egg production, however, which can only be explained by the occurrence of two or more ovulations at unusually short intervals. Such cases are seen when double-yolked eggs are produced on successive days, and when a double-yolked egg is laid after a long series of normal daily eggs. Several records of this kind have been obtained from the station flock.

Triple-Yolked Eggs.

The same processes which cause the formation of double-yolked eggs may also cause the formation of triple-yolked eggs. The number of triple-yolked eggs, however, is so small that an extensive study of them is impossible. The first of the triple-yolked eggs produced by the station flock was fully described by Dr. Raymond Pearl.[1] He called attention to the size relation between this egg and double-yolked, single-yolked and yolkless eggs of the same breed (Barred Plymouth Rock), and showed that the parabola

$$W = 16.242 + 43.762\,Y - 4.450\,Y^2$$

gave an extremely close fit to the observations.

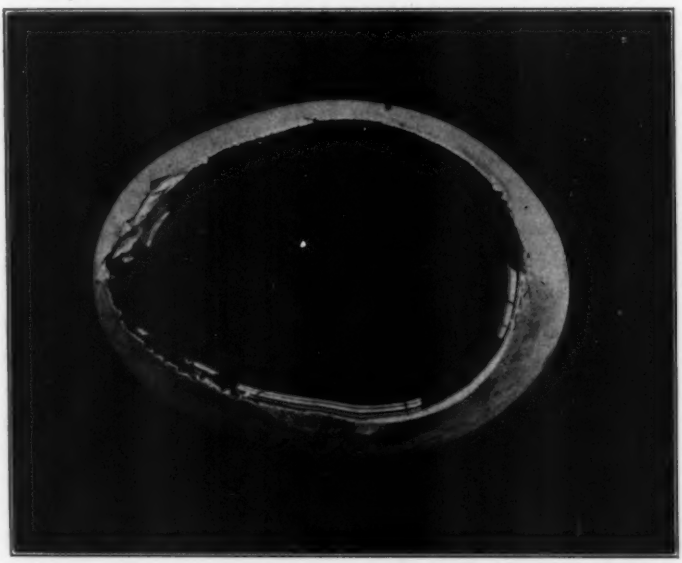


Fig. 2. Triple-yolked egg.

He describes the internal characters of the egg as follows: "Each yolk was enclosed in a separate yolk membrane. While the three yolks were in contact with each other, they were in no

[1] Pearl, R., "A Triple-Yolked Egg," *Zool. Anz.*, Bd. XXXV., pp. 418–423.

way fastened together. All of the yolks were of normal size, and of approximately the same size . . . the two kinds of albumen (of thick and thin consistency) which are normal were present in this egg. There was no trace of a chalaza in connection with any yolk or at either pole of the egg. The shell membranes were entirely normal."

The second triple-yolked egg was produced September 16, 1913, by bird No. 65K. The egg appeared externally like a large double-yolked egg. Its dimensions were as follows:

Length—63.6 mm.
Breadth—47.6 mm.
Weight—79.62 gms.

This was the first egg laid by the young pullet and was much smaller than the egg described by Pearl.

A photograph of the internal arrangement of the egg parts is shown in Fig. 2.

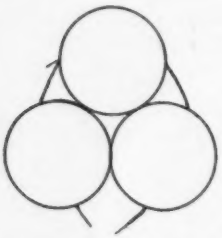


FIG. 3. Showing the arrangement of yolks in the triple-yolked egg.

The three yolks lay close together in a common thick albumen envelope. The chalazæ were feebly developed but their relations are shown in Fig. 3.

The yolks evidently entered the oviduct at successive short intervals and came down the funnel region in "Indian file". There was a short end of chalazal fibers at the caudal end of the second yolk but other fibers were continued to the third yolk as shown in the figure. Since the three yolks were close together in a common thick albumen envelope they must have joined at or very near the anterior end of the albumen secreting region.

Normal thin albumen and a normal shell membrane and shell were present.

The weights of the parts of this egg were as follows:

Weight of yolks	9.61 gms.	
	9.45 gms.	
	9.22 gms.	
	28.28 gms.	Percentage of yolk 35.52
Weight of albumen	44.24 gms.	Percentage of albumen 55.56
Weight of shell	7.10 gms.	Percentage of shell 8.92

The third triple-yolked egg was produced Oct. 8, 1913, by bird No. 16K. It was her sixth egg. The dimensions were as follows:

Length—65.2 mm.
Breadth—46.2 mm.
Weight—77.41 gms.

The egg was opened and the three yolks were seen lying in practically the same position as those in the triple-yolked egg of 65K. In an attempt to get a photograph of this egg the edge of the shell was accidently pressed against two of the yolks and yolk flowed out into the albumen. The yolk which was not broken and the shell were weighed. More data could not be accurately obtained but on the assumption that the yolk saved represented a mean of the three yolks the data for this egg are as follows. (The calculated data are in italics.)

Weight of yolk	= 10.29 × 3 = *30.87* gms.	Percentage of yolk.....	*39.88*
Weight of albumen	= *30.17* gms.	Percentage of albumen..	*50.60*
Weight of shell	= 7.37 gms.	Percentage of shell.....	9.52

The Size and Proportion of Parts in the Single-, Double- and Triple-Yolked Eggs of the Same Individual.

On September 28 and 29 the bird 65K which had laid the second triple-yolked egg laid normal single-yolked eggs. These eggs were not preserved. On October 2 she laid a double-yolked egg. Complete data were taken on this egg.

It was apparent that this bird's eggs offered an excellent opportunity to study the proportion of the parts in multiple eggs when compared with normal eggs produced by the same individual. Accordingly data were taken on the next ten normal eggs. On October 6 the bird produced another double-yolked egg. This was her seventh egg. Both of the double-yolked eggs of this bird had separate chalazal membranes but a common thick albumen envelope.

The data on the triple- and double-yolked eggs and the mean for the ten normal eggs are given in Table II.

The data given in this table show that the double-yolked eggs of this bird are not twice, nor the triple-yolked eggs three times, the size of the normal single-yolked eggs. This agrees with

TABLE II.

SHOWING THE DATA ON THE TRIPLE-YOLKED EGG, THE TWO DOUBLE-YOLKED EGGS AND THE MEAN OF TEN NORMAL EGGS OF 65K.

Egg No.	Date.	Length, Mm.	Breadth, Mm.	Index, Per Cent.	Weight of Egg, Gm.	Weight Albumen, Gm.	Per Cent. Albumen.	Weight Yolk, Gm.	Per Cent. Yolk.	Weight Shell, Etc., Gm.	Per Cent. Shell, Etc.
1	Sept. 26	63.6	47.6	74.84	79.62	44.24	55.56	9.61 9.45 9.22 28.28	35.52	7.10	8.92
4	Oct. 2	60.0	44.4	74.00	66.48	37.28	56.08	11.10 11.60 22.70	34.15	6.50	9.78
7	Oct. 6	59.7	45.5	76.21	69.67	39.05	56.05	11.80 11.65 23.45	33.66	7.17	10.29
Mean of double-yolked eggs.		59.85	44.95	75.11	68.08	38.17	56.07	23.08	33.91	6.84	10.04
Mean of 10 normal eggs.	Oct. 3 to Oct. 17	52.29	40.52	77.53	49.11	31.23	63.58	11.96	24.37	5.92	12.05

Pearl's[1] conclusion "that the size of eggs is not directly proportional to the number of yolks they contain." The data on which he based his conclusion were taken from the eggs of different individuals of various ages and the eggs were produced at different seasons of the year. Since all these factors influence the size and the proportion of parts of the egg it is interesting to note that his conclusion is confirmed by the present observation, made on eggs of a single individual laid in a period of three weeks' duration.

The data given in Table II. also show that the different parts of the double- and triple-yolked eggs are in different proportions than in single-yolked eggs. Fig 4 shows graphically for the eggs of 65K the mean weight of the egg and of each egg part for eggs with one, two and three yolks.

Both the diagram and the table show that the weight of albumen and shell are increased in double-yolked eggs. These components still further increase in triple-yolked eggs, but the increase in these parts is less than the increase in yolk weight. This may be seen either from the apparent slope of the lines in the figure, or from a comparison of the actual weight of the parts

[1] *Loc. cit.*

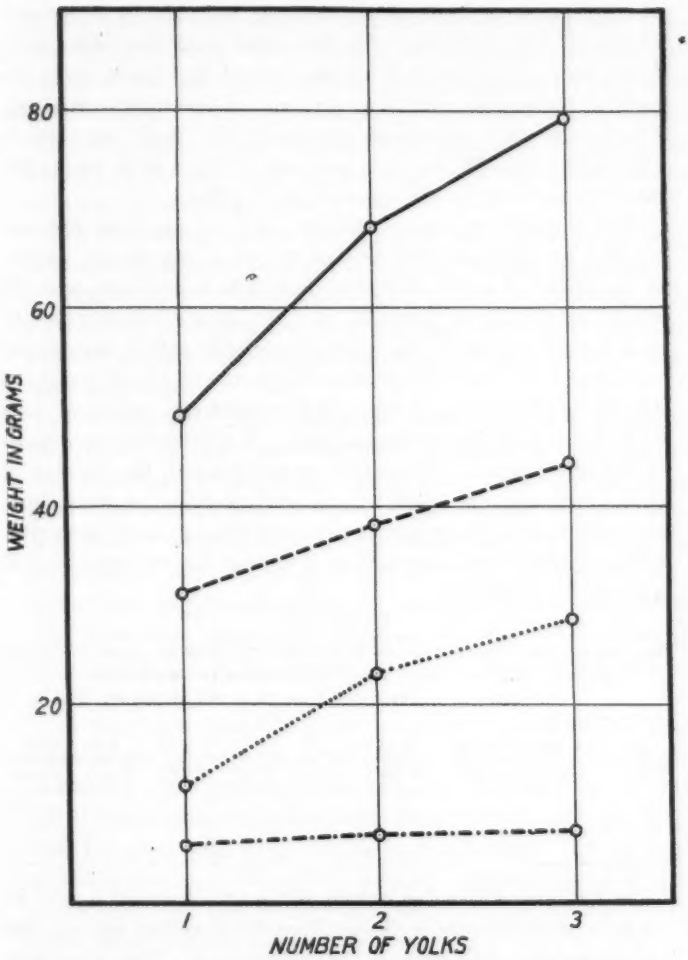


FIG. 4. Showing the mean weight in grams of the egg and each egg part for single-, double-, and triple-yolked eggs of 65K. Solid line = egg weight. Dash line = albumen weight. Dot line = yolk weight. Dash and dot line = shell weight.

in the different sorts of eggs, or from a comparison of the percentages. A comparison of the percentages shows that albumen forms 63.58 per cent. of the normal eggs of this bird, but only

56.07 per cent. of the double-yolked egg and only 55.56 per cent. of the triple-yolked egg. On the other hand the yolk, which forms only 24.37 per cent. of the normal egg, forms 33.91 per cent. of the double, and 35.52 per cent. of the triple-yolked egg. Finally the shell percentage decreases from 12.05 per cent. in eggs with one yolk, to 10.04 per cent. in eggs with two yolks, and 8.92 per cent. in the egg with three yolks.

The different relation of the egg parts in eggs with different numbers of yolks may also be seen by comparing the egg weight, or the weight of a part, in double- and triple-yolked eggs, with the weight of the egg or the same part of a single-yolked egg. If, as is usually assumed, the yolks of multiple-yolked eggs are of the same size as the yolks of normal eggs, the total yolk weight of double- and triple-yolked eggs will be respectively twice and three times the yolk weight of normal eggs. If in addition the weights of the other parts were directly proportional to the number of yolks contained, the weight of each part would be as many times the weight of the same part of the normal egg as there are yolks. That neither of the assumptions is correct for the eggs of 65K is shown by Table III.

TABLE III.

SHOWING FOR THE EGGS OF 65K THE RATIO THAT THE WEIGHT OF THE WHOLE OR OF EACH PART OF A MULTIPLE-YOLKED EGG IS OF THE WEIGHT OF THE WHOLE OR THE SAME PART OF A SINGLE-YOLKED EGG.[1]

No. of Yolks in the Egg.	Ratio of the Egg Weight to the Weight of a Single-yolked Egg.	Ratio of the Yolk weight to the Yolk weight of a Single-yolked Egg.	Ratio of the Albumen Weight to the Albumen Weight of a Single-yolked Egg.	Ratio of Shell Weight to the Shell Weight of a Single-yolked Egg.
2	1.38	1.93	1.22	1.16
3	1.62	2.36	1.42	1.20

The figures in this table as well as the actual weight figures in Table II. show that the yolks in the multiple-yolked eggs of this bird are smaller than those in the normal eggs. They also show that the weights of albumen and shell are not as many times the weight of the same parts in the normal egg as is the weight of the yolk. The fact that the yolks of the double-yolked eggs were smaller than the yolks of the single-yolked eggs, and that the yolks of the triple-yolked eggs were still smaller, is in this case

[1] The mean of the ten single-yolked eggs given in Table II.

probably due entirely to the fact that the multiple-yolked eggs were laid just as the bird was coming to sexual maturity and thus preceded practically all the normal eggs with which they are compared. The triple-yolked egg was the first egg laid by this young pullet and the double-yolked eggs were the fourth and seventh, while the single yolked eggs were the fifth and the eighth to sixteenth inclusive.

The effect of the maturity of the bird upon the size of the egg and especially upon the weight of the yolk has been discussed in a previous paper.[1] The question now arises as to whether in general the yolks of multiple-yolked eggs are smaller than the yolks of single-yolked eggs produced at the same period by the same individual.

In the course of the investigation referred to above some data bearing on this point were obtained. Five of the birds used in this investigation produced one or more double-yolked eggs. Data were taken on all of the eggs laid by each of these birds. It is thus possible to compare the actual size and the size and proportion of the parts of each double-yolked egg with the mean of the normal egg produced by the same bird at the same period. The mean was, therefore, calculated for the ten eggs which succeeded each double-yolked egg. These data are brought together in Table IV.

In this table data are given on eleven double-yolked eggs. Four of the eleven were either first or second eggs of a young pullet and are, therefore, of no value in the present connection. In all these, as would be expected, the weight of each yolk is less than the mean yolk weight of the succeeding eggs. Of the seven double-yolked eggs which were not first or second eggs, four have yolks which are practically the same size or larger, while three have yolks that are significantly smaller.

The size of each yolk of double-yolked eggs is probably in general not any smaller than that of the normal eggs produced by the same individual at the same period of production. When multiple-yolked eggs are among the first pullet eggs, however, the

[1] Curtis, M. R., "A Biometrical Study of Egg Production in the Domestic Fowl, IV., Factors Influencing the Size, Shape and Physical Constitution of Eggs." (In press.)

TABLE IV.

THE DIMENSIONS, SHAPE, WEIGHT AND THE WEIGHT AND PROPORTION OF PARTS OF EACH DOUBLE-YOLKED EGG AND THE MEAN OF THE TEN SUCCEEDING SINGLE-YOLKED EGGS PRODUCED BY A FLOCK OF BARRED PLYMOUTH ROCK BIRDS DURING THEIR FIRST TWO LAYING YEARS.

Bird No.	Egg No.	Date.	Length, Mm.	Breadth, Mm.	Index, Per Cent.	Weight of Egg, Gm.	Weight of Albumen, Gm.	Per Cent Albumen.	Weight Yolk, Gm.	Per Cent. Yolk.	Weight Shell, Etc., Gm.	Per Cent. Shell.
218	7	Nov. 19, 1910.	65.60	43.61	66.48	73.27	40.84	55.74	12.73 11.78 24.51	33.45	7.91	10.81
218	Mean of 10 following eggs		51.78	39.42	75.87	45.95	27.86	60.62	12.63	27.49	5.47	11.90
218	239	Feb. 25, 1912.	70.90	46.30	65.30	87.20	47.58	54.56	17.04 15.01 32.05	36.75	7.57	8.68
218	Mean of 10 following eggs		60.00	42.01	70.06	59.04	33.19	56.18	19.81	33.58	6.04	10.22
218	342	Aug. 13, 1912.	76.20	46.36	60.84	92.71	48.32	52.12	18.64 18.57 37.21	40.14	7.18	7.74
218	Mean of 10 following eggs		57.20	41.16	72.03	54.07	31.19	57.69	17.46	32.29	5.42	10.03
218	350	Sept. 9, 1912.	74.30	47.70	64.20	91.97	46.21	50.24	19.34 18.97 38.31	41.65	7.45	8.10
218	Mean of 10 following eggs		59.34	42.06	70.96	57.58	32.88	57.11	18.92	32.85	5.78	10.04
218	Minimum for all normal 1st year eggs				67.40							
139	1	Oct. 27, 1910.	71.77	42.15	58.73	77.41	45.10	58.26	11.51 12.26 23.77	30.71	8.54	11.03
139	Mean of 10 following eggs		54.38	40.34	74.22	50.49	30.00	59.37	14.37	28.50	6.12	12.13

TABLE IV.—*continued.*

Bird No.	Egg No.	Date.	Length, Mm.	Breadth, Mm.	Index, Per Cent.	Weight of Egg, Gm.	Weight of Albumen, Gm.	Per Cent. Albumen.	Weight Yolk, Gm.	Per Cent. Yolk.	Weight Shell, Etc., Gm.	Per Cent. Shell.
139	129	June 22, 1911.	71.80	44.10	61.42	80.21	45.05	56.17	14.11 13.91 28.02	34.93	7.14	8.90
139	Mean of 10 following eggs		57.87	41.90	72.43	57.17	35.36	61.83	16.28	28.49	5.53	9.68
139	160	Aug. 17, 1911.	68.00	45.75	67.28	85.97	47.35	55.08	16.08 15.37 31.45	36.58	7.17	8.34
139	Mean of 10 following eggs		58.16	42.33	72.82	59.03	36.93	62.59	16.29	27.57	5.81	9.84
130	Minimum for all normal 1st year eggs				63.87							
478	1	Nov. 7, 1910.	69.10	44.03	63.72	74.85	48.79	65.18	9.30 9.64 18.94	25.30	7.12	9.51
478	2	Nov. 9, 1910.	64.70	44.12	68.20	72.29	43.35	59.97	10.57 10.80 21.37	29.56	7.57	10.47
478	Mean of 10 following eggs		52.78	41.22	78.19	51.30	34.08	66.34	11.58	22.66	5.65	11.01
478	Minimum for all normal 1st year eggs				69.26							
192	2	Nov. 5, 1910.	60.85	39.60	65.08	54.77	27.38	49.99	10.09 10.94 21.03	38.40	6.36	11.61
192	Mean of 10 following eggs		52.34	37.65	71.94	42.71	24.99	58.52	12.87	30.12	4.85	11.36
192	Minimum for all normal 1st year eggs				69.10							
204	179	Feb. 12, 1912.	72.50	44.92	61.96	84.10	45.85	54.52	15.41 16.42 31.83	37.85	6.42	7.63
204	Mean of 10 following eggs		60.67	41.19	67.90	58.89	34.43	58.44	18.68	31.72	5.79	9.84
204	Minimum for all normal 1st year eggs				65.73							

yolks are smaller than those of succeeding eggs since at this period the successive yolks are increasing in size.

Table IV. shows also that in all cases the absolute weight of both albumen and shell is higher in double-yolked eggs than in the mean of the next ten normal eggs, but in none of the double-yolked eggs observed was the percentage of either albumen or shell as high as in the succeeding normal eggs. These observations are in agreement with those on the eggs of 65K (see Table II.). They show that while an increase in the stimulation causes an increase in the amount of secretion the increase is not in direct proportion to the weight of the substance causing the stimulation.

This may be due to the fact that the effective stimulation is not directly proportional to the weight. That is, it may be due to the size of the area of contact, or to the time the stimulus is applied to each successive area of the secreting membrane. Or again it may be due to a deficiency of material available for the formation of the secretion, or of time necessary for its elaboration.

In this connection attention should be called to two facts noted in previous paragraphs, first that when the eggs have separate chalazal membranes the chalazæ of the second yolk are usually much less developed than the first, and second that when the eggs have separate thick albumen envelopes the one surrounding the second yolk is more viscous but forms a thinner layer. In such cases at least the two yolks represent successive stimulations rather than a single intensified stimulation, and the glands are evidently not in a condition to respond in the same degree to the second stimulation. There is, of course, the possibility that the difference in the quantity and quality of secretion in the two cases may in part be due to the fact that the second yolk is moved more rapidly than the first but this theory would not account for the smaller per cent. of albumen in double-yolked eggs where the two yolks have common albumen envelopes.

The processes which determine the quantitative relationship between the yolk and albumen and between yolk + albumen and shell are too complex for solution with our present knowledge. Yet it is certain that although practically doubling and

tripling the weight of yolks does not cause a proportionate increase in the secretion of albumen and shell, nevertheless doubling causes an increase and tripling a still slightly greater increase. This shows that either the glands of the oviduct are not all completely discharged during the formation of a single-yolked, or even a double-yolked egg, or that under stimulation they elaborate their secretions with great rapidity.

A Comparison between the Shape of Single- and Multiple-Yolked Eggs of the Same Individual.

It may be seen from Tables II. and IV. that there is a decided difference in the shape of single- and multiple-yolked eggs produced by the same bird. The multiple-yolked eggs are both longer and broader than the single-yolked eggs, but they are not as broad in proportion to their length. This may be best seen by comparing the length-breadth index $\frac{100 \text{ breadth}}{\text{length}}$ of the double-yolked or triple-yolked egg with the mean index for the ten succeeding eggs. In all cases the mean index for the normal eggs is higher, showing that the normal egg is broader in proportion to its length. In Table IV. the lowest index for the eggs produced by each bird during the first year is also given. In every case but one the index of the double-yolked egg is lower than the minimum for all the first year normal eggs. That is, it falls below the normal variation for the individual. The multiple-yolked egg then is longer in proportion to its breadth than any of the normal eggs of the same individual.

The yolks pass down the duct in succession, and not abreast. In the complete egg they lie with their common axis in the long axis of the egg. They must, therefore, stimulate the oviduct glands in succession. However, the eggs with two or three yolks are actually broader than those with one. This may be due in part to the fact that the field of stimulation of the two yolks overlaps, but probably the most important factor is the mechanical one due to forcing (by peristalsis) a larger plastic mass through an elastic tube, which offers more resistance to the passage of a large than a small body.

Summary.

1. During the last six years more than three thousand different domestic fowls, which have been kept at least one year at the Maine Agricultural Experiment Station, have laid but three triple-yolked eggs.

2. Each of these eggs was laid by a different individual and in each case the triple-yolked egg was one of the first eggs produced by a young pullet.

3. Young pullets also show a decided tendency to produce double-yolked eggs when they first begin to lay. About 20 per cent. of the pullets which lay before they are seven months old lay among their first eggs one or more with two yolks.

4. Nearly 80 per cent. of the individuals of the flock never lay a double-yolked egg.

5. Mature birds also sometimes produce double-yolked eggs; but most such birds have also produced one or more when they were young pullets.

6. There has been no bird in the experiment station flock with which the laying of double-yolked eggs was "habitual" although there are some which have produced several such eggs.

7. The production of an egg with two or three yolks represents the extreme of rapid egg production, other forms of which are found in the production of two eggs united by a membranous tube; two eggs at the same time; two eggs at different times on the same day and a daily egg production where the eggs are laid earlier on each successive day.

8. The two yolks of a double-yolked egg may have all the egg envelopes in common, indicating that they have passed the entire length of the duct together; or each may possess one or more separate envelopes. There are also all the possible intermediate forms indicating that the two yolks in a common shell may unite at any point between the mouth of the funnel and the isthmus. When two eggs come together after the first has entirely passed the anterior end of the isthmus the result is the production of two eggs at the same time.

9. Various disturbances of the normal processes of egg production may bring two yolks together in the oviduct. Double-yolked eggs evidently do not always represent simultaneous

ovulations. The assumption of simultaneity or abnormally close succession of ovulations is necessary to account for the production of a succession of double-yolked eggs or of a double-yolked egg immediately following a long series of normal daily eggs.

10. The double-yolked eggs contain more albumen and have a heavier shell than single-yolked eggs, and in triple-yolked eggs these parts are heavier than in double-yolked eggs. Yet these parts do not increase in direct proportion to the increase in the weight of yolk. That is, the percentage of albumen and shell is less in double- than in single-yolked eggs and is still smaller in triple-yolked eggs.

11. The yolks of the multiple-yolked eggs of mature birds are not consistently smaller than the yolks of the normal eggs produced during the same period.

12. Multiple-yolked eggs are longer in proportion to their breadth than the normal eggs of the same individual.

The physiolcgical bearing of these facts is discussed.

THE CHANGE IN VOLUME OF ARBACIA AND ASTERIAS EGGS AT FERTILIZATION.[1]

OTTO GLASER.

In my paper on the initiation of development, Glaser (13), I stated that the eggs of *Arbacia punctulata* and *Asterias forbesii* are smaller in volume after fertilization than before. In the pages that follow I wish to present the evidence for this assertion in some detail, since for the present at least, it must stand alone. *Strongylocentrotus* (*purpuratus?*) shows no recognizable loss of volume according to Loeb ('08) but this result does not contradict mine. The loss depends on several conditions, and in *Asterias* is much greater than in *Arbacia*. This suggests the possibility of forms in which it may easily be too small to measure. The measurements of McClendon ('10), also, do not contradict mine, although they seem to show that the very eggs I worked with, on fertilization lose in a molar solution of dextrose, but remain constant, or even gain a little in sea water. The evidence consists in the determination of the diameters of 19, 20, 10 and 11 eggs respectively. The great variability of the ova of *Arbacia punctulata*, however, proves that reliable results can be gotten from a small number of eggs, only when the identical ones are measured before and after fertilization.

Careful tracings were made at known magnifications with the aid of a camera lucida, and a crow-quill pen, on paper of known weight. On the assumption that the eggs are spheres, and that one is really drawing their great circles, the areas of the outlines obtained in this manner, were first determined by means of a planimeter, and afterwards by carefully cutting them out and weighing the discs. For enlargements of 740 diameters both methods gave identical results, but for the smaller magnifications the method of weighing proved more reliable. For this reason, in the comparisons of fertilized with unfertilized eggs, only data

[1] From the Marine Biological Laboratory at Woods Hole, and Zoölogical Laboratory of the University of Michigan.

obtained by the latter method are reported, whereas the variability curves are based on factored measurements by the planimeter. The same curves were first constructed from the original measurements.

On the Constancy in Volume of the Eggs of a Single Female.

In Table I. are given the diameters of 280 different eggs derived from one female, measured in groups of ten, at three distinct periods after removal from the ovaries. The results show not only a remarkable constancy for the ova of a single individual, when grouped in this manner, but what is of even more immediate importance, they show that in four hours, the eggs do not undergo any change in volume discoverable by the methods employed.

Table I.

Female B.

10 eggs, 4.35 P.M. 81.4 micra.	5.40 P.M. 82.6 micra.	8.35 P.M. 79.6 micra.
10 eggs, 4.35 P.M. 80.0 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 81.6 micra.
10 eggs, 4.35 P.M. 81.2 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 79.6 micra.
10 eggs, 4.35 P.M. 80.6 micra.	5.40 P.M. 79.4 micra.	8.35 P.M. 79.0 micra.
10 eggs, 4.35 P.M. 82.4 micra.	5.40 P.M. 78.6 micra.	8.35 P.M. 81.0 micra.
10 eggs, 4.35 P.M. 80.6 micra.	5.40 P.M. 81.6 micra.	8.35 P.M. 82.4 micra.
10 eggs, 4.35 P.M. 78.8 micra.	5.40 P.M. 81.2 micra.	8.35 P.M. 82.2 micra.
10 eggs, 4.35 P.M. 81.4 micra.	5.40 P.M. 80.8 micra.	8.35 P.M. 81.0 micra.
10 eggs, 4.35 P.M. 78.2 micra.	5.40 P.M. 77.4 micra.	8.35 P.M. 80.0 micra.
10 eggs, 4.35 P.M. 83.6 micra.	5.40 P.M. micra.	8.35 P.M. micra.
Average 80.8 micra.	Average 80.4 micra.	Average 80.6 micra.

On the Volume of the Eggs of Different Females.

To what extent the average volume of the ova of different females is constant, I am unable to say, since scarcely anything much less than one hundred eggs from each would suffice to decide the question. One would not expect great constancy however unless the numerous factors entering into the result were identical. In the one case in which I have the requisite number of measurements for such comparison, the average diameter is the same as that found for female B.

Table II.

Female C.

10 eggs 80.4 diam.
10 eggs 82.2 diam.
10 eggs 80.2 diam.
10 eggs 79.6 diam.
10 eggs 81.6 diam.
10 eggs 79.0 diam.
10 eggs 77.8 diam.
10 eggs 79.2 diam.
10 eggs 79.2 diam.
Average 80.0 diam.

The Diameters of Individual Eggs.

In the two preceding tables the measurements are given for groups of ten eggs each. Comparison of the various sets indicates considerable variability, but the true extent of this can only be realized when a large number of ova is treated individually. This was done with 100 eggs of female B and 100 of female C. The results are given in Tables III. and IV.

Table III.

Female B, Individual Eggs.

Of 100 eggs 1 had a diameter of 49.8 micra.		
Of 100 eggs 1 had a diameter of 58.8 micra.	Average of 3 = 57.1	
Of 100 eggs 1 had a diameter of 61.6 micra.		
Of 100 eggs 1 had a diameter of 64.2 micra.		
Of 100 eggs 1 had a diameter of 65.4 micra.	Average of 3 = 65.5	
Of 100 eggs 1 had a diameter of 66.8 micra.		
Of 100 eggs 2 had a diameter of 68.0 micra.		
Of 100 eggs 4 had a diameter of 70.6 micra.	Average of 11 = 70.8	
Of 100 eggs 5 had a diameter of 72.0 micra.		
Of 100 eggs 5 had a diameter of 73.2 micra.		
Of 100 eggs 3 had a diameter of 74.6 micra.	Average of 16 = 74.2	
Of 100 eggs 8 had a diameter of 76.0 micra.		
Of 100 eggs 3 had a diameter of 77.2 micra.		
Of 100 eggs 5 had a diameter of 79.6 micra.	Average of 15 = 79.2	
Of 100 eggs 7 had a diameter of 79.8 micra.		
Of 100 eggs 8 had a diameter of 81.2 micra.		
Of 100 eggs 2 had a diameter of 82.4 micra.	Average of 20 = 82.6	
Of 100 eggs 10 had a diameter of 83.8 micra.		
Of 100 eggs 7 had a diameter of 85.0 micra.		
Of 100 eggs 4 had a diameter of 86.4 micra.	Average of 14 = 84.5	
Of 100 eggs 3 had a diameter of 87.6 micra.		
Of 100 eggs 6 had a diameter of 89.0 micra.		

TABLE III. *Continued.*

FEMALE B. INDIVIDUAL EGGS.

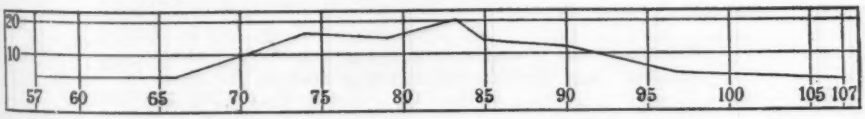
Of 100 eggs 3 had a diameter of 90.4 micra.	Average of 12 = 90.0
Of 100 eggs 3 had a diameter of 91.6 micra.	
Of 100 eggs 2 had a diameter of 94.2 micra.	
Of 100 eggs 1 had a diameter of 96.8 micra.	Average of 4 = 96.5
Of 100 eggs 1 had a diameter of 100.8 micra.	
Of 100 eggs 1 had a diameter of 102.0 micra.	
Of 100 eggs 1 had a diameter of 112.6 micra.	Average of 2 = 107.3

TABLE IV.

FEMALE C, INDIVIDUAL EGGS.

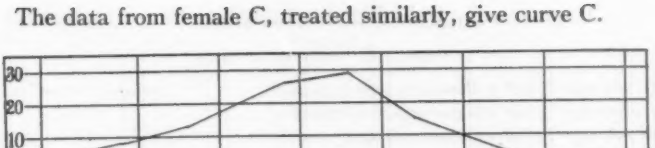
Of 100 eggs 1 had a diameter of 59.4 micra.	
Of 100 eggs 1 had a diameter of 65.2 micra.	Average of 3 = 63.7
Of 100 eggs 1 had a diameter of 66.6 micra.	
Of 100 eggs 2 had a diameter of 68.0 micra.	
Of 100 eggs 5 had a diameter of 69.6 micra.	Average of 8 = 69.4
Of 100 eggs 1 had a diameter of 71.0 micra.	
Of 100 eggs 5 had a diameter of 72.2 micra.	
Of 100 eggs 4 had a diameter of 73.8 micra.	Average of 13 = 72.9
Of 100 eggs 4 had a diameter of 75.4 micra.	
Of 100 eggs 6 had a diameter of 76.8 micra.	
Of 100 eggs 9 had a diameter of 78.2 micra.	Average of 26 = 78.5
Of 100 eggs 11 had a diameter of 79.6 micra.	
Of 100 eggs 8 had a diameter of 81.0 micra.	
Of 100 eggs 11 had a diameter of 82.6 micra.	Average of 29 = 82.6
Of 100 eggs 10 had a diameter of 83.8 micra.	
Of 100 eggs 7 had a diameter of 85.6 micra.	
Of 100 eggs 4 had a diameter of 87.0 micra.	Average of 15 = 86.7
Of 100 eggs 4 had a diameter of 88.4 micra.	
Of 100 eggs 2 had a diameter of 89.8 micra.	
Of 100 eggs 1 had a diameter of 94.2 micra.	Average of 4 = 92.4
Of 100 eggs 1 had a diameter of 95.6 micra.	
Of 100 eggs 1 had a diameter of 100.0 micra.	Average of 1 = 100.0

If one treats the diameters and eggs in groups of three, and plots the latter on the ordinate and the former on the abscissa the following curve emerges for female B.



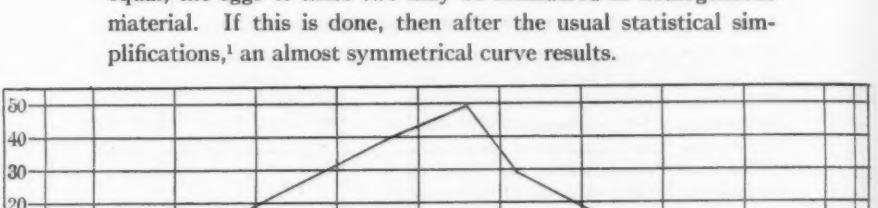
CURVE B. Diameters of 100 eggs from one specimen of *Arbacia punctulata*.

The data from female C, treated similarly, give curve C.



CURVE C. Diameters of 100 eggs from one specimen of *Arbacia punctulata*.

Since the diameters for females B and C on the average are equal, the eggs of these two may be considered as homogeneous material. If this is done, then after the usual statistical simplifications,[1] an almost symmetrical curve results.



CURVE BC. A composite of the diameters of 200 eggs from two specimens of *Arbacia punctulata*.

COMPARISON OF UNFERTILIZED AND FERTILIZED OVA.

The variability exhibited by these eggs and the differences in range between different females, show clearly that if one wishes to make trustworthy comparisons between the volumes of unfertilized and fertilized ova, it is either necessary to use a large number of each kind from a single female or to deal with a smaller number and measure the identical eggs before and after fertilization.

Before adopting the second alternative, I made a series of tracings of unfertilized and fertilized ova to see whether measureable differences really existed. The results, averaged, were as follows:

Unfertilized eggs; 77 measurements; average diameter, 74.1 micra.
Fertilized eggs; 91 measurements; average diameter, 70.7 micra.
Loss 3.4 micra.

[1] The eggs are treated in sets of three, and average diameters not more than three micra apart are considered as belonging to the same group.

This result, further discussion of which is unnecessary, encouraged me to undertake a more careful study of a few eggs. Although involving more labor, this alternative was chosen in preference to the "wholesale" method, because the behavior of individual eggs could be definitely recorded. The probable advantage of this was indicated by the discovery by Glaser ('13) that the number of sperm used in insemination plays a rôle in the result. Accordingly "wholesale" measurements, which are satisfactory enough to decide whether or not a determinable change in volume occurs, have the disadvantage of covering up the fact that the loss is by no means a constant quantity.

In making these particular measurements, special precautions were taken. The eggs were traced one at a time, no other eggs were on the slide, and the focus before and after fertilization remained unchanged. This was not easy to accomplish, for the sperm are apt to set the egg in motion and change its position. Whenever this occurred, the attempt to secure the second measurement was abandoned. Naturally only a small number of cases could be collected under these conditions.

In order to fulfill the aforesaid requirements, and keep the magnification of the tracings absolutely constant, it was necessary to use small quantities of sperm. This however introduced other difficulties. The appearance of the fertilization membrane is the only convenient index of impregnation, but the membrane does not appear unless the spermatozoa are present in sufficient numbers. As a consequence, I often failed to observe the only available indication of fertilization.

While the results which I have seem to me to point in but one direction, the changes in volumes registered are in most cases probably minimal, for they depend on the amount of surface alteration, and this in turn is, within limits, a function of the number of sperm involved. Indeed with great excesses of sperm I have not infrequently observed a destructive alteration of the surface of the egg. This is more easily observable in *Asterias* than in *Arbacia*. It appears likely therefore that normally, or perhaps better, frequently, greater changes in volume occur than I have recorded.

The results on individual eggs dealt with singly in the manner described are given in the subjoined table.

TABLE V.

INDIVIDUAL EGGS BEFORE AND AFTER FERTILIZATION.

Female.	Egg.	Measurements.	Before.	Measurements.	After.	Change in diameter.
D_1	1	3	81.2	2	75.4	−5.8
D_1	2	3	80.6	3	68.8	−11.8
D_1	3	3	73.4	3	65.4	−8.0
D_1	4	3	78.8	3	72.8	−6.0
D_1	5	3	74.6	3	68.4	−6.2
D_2	1	4	70.0	3	66.6	−3.4
D_2	2	4	71.2	4	67.2	−4.0
D_2	3*	3	78.0	4	78.2	+0.2*
D_2	4	4	75.2	3	73.4	−1.8
D_2	5	3	78.0	4	75.2	−2.8
E	1	3	70.0	3	68.2	−1.8
E	2*	4	65.2	2	65.8	+0.6*
E	3	3	72.2	4	65.6	−6.6
E	4	3	72.2	4	69.4	−2.8
E	5	3	68.8	4	67.8	−1.0

With the exception of the starred eggs, D_2 3, and E 2, which within the limits of error may easily be considered constant, all the eggs showed a noticeable decrease.

The fact brought out by these measurements constitutes an additional support for the conception that an increase in the permeability of the egg accompanies fertilization. With such increase, other conditions remaining constant, one would expect a change in volume. The fact that unfertilized eggs, under certain conditions, may remain unaltered for hours, whereas there is a noticeable decrease in their diameters immediately after fertilization, strongly emphasizes the change that takes place.

COMPARISON OF UNFERTILIZED AND FERTILIZED OVA OF *Asterias*.

The results obtained in the case of *Arbacia* are more strikingly illustrated by a control observation made on the ova of *Asterias forbesii*. The measurements were made on eggs which had been removed from the ovaries and allowed to stand one hour in sea water in order to complete maturation. Tracings were made of two small groups from two females and these groups were then inseminated. It is impossible to identify individual eggs in this case, but this does not invalidate the result, since no eggs were either added or removed upon the addition of the sperm. Seventeen eggs derived from one female, and eight from the other

were used, and the same eggs were traced after fertilization that had been traced before. The result was:

Female 1; 17 eggs { Diameter before fertilization, 103.6 micra. / Diameter after fertilization, 85.2 micra. } Loss 18.4 micra.

Female 2: 8 eggs { Diameter before fertilization, 105.0 micra. / Diameter after fertilization, 94.0 micra. } Loss 11.0 micra.

These values cannot be considered standard figures any more than those found in the case of the sea urchin, yet they seem to indicate with certainty that the egg of the starfish also loses in volume on fertilization. In fact the loss recorded is much greater than that found for *Arbacia* eggs. A portion of this difference may be due to unequal amounts of sperm used in the two cases, but the entire variation can hardly be accounted for on this basis. Normally the perivitelline space of fertilized *Asterias* eggs is proportionately larger than in *Arbacia*. If the space in question comes about as Loeb ('08) has contended, when a colloidal substance secreted by the egg absorbs water, then on the assumption that this material has essentially the same properties in *Arbacia* and in *Asterias*, the egg having the greater vitelline space should secrete the greater quantity, and therefore decrease correspondingly more in volume. That the *Asterias* eggs measured lost more on the average than the *Arbacia* treated either en masse, or as individuals, can be seen by a glance at the tables. One is led to suspect from these considerations that the surface of the *Asterias* egg is more easily modifiable than that of the *Arbacia* ovum. This is in harmony with the well known experience that artificial parthenogenesis can be induced in *Asterias* by a greater variety of methods than in *Arbacia*.

UNIVERSITY OF MICHIGAN,
November 7, 1913.

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Loeb, Jacques

'08 Über die Osmotischen Eigenschaften und die Entstehung der Befruchtungsmembran beim Seeigelei. Archiv. f. Entwicklungsmechanik, Bd. 26.

McClendon, J. F.

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VOLUMETRIC CHANGES IN THE EGG OF THE BROOK LAMPREY, ENTOSPHENUS (LAMPETRA) WILDERI (GAGE), AFTER FERTILIZATION.[1]

PETER OKKELBERG.

Various investigators have found by actual measurements that the eggs of certain animals do not change appreciably in volume as a result of fertilization. Calberla[2] in his work on the fertilization process in the egg of *Petromyzon planeri* says: "Genaue Messungen mittelst des Zeichnenprismas (Oberhäuser), die während des Befruchtungsvorganges angestellt wurden, erwiesen es als zweifellos, dass der Dotter sich nicht contrahirt, dagegen die Eihaut sich enorm ausdehnt. . . . Allerdings nimmt der Dotter, nachdem er von der Eihautberührung befreit worden ist, eine andere Form an, d. h. er geht von der Ellipsoidform in die Kugelform über, aber dies geschieht ohne Volumsverminderung." McClendon,[3] working on the eggs of *Arbacia punctulata*, found that when the eggs were placed in a molecular solution of cane sugar, approximately isosmotic with sea water, the mean diameter of a certain number of eggs was 83 when not fertilized and 75 when fertilized. Control eggs in ordinary sea water showed mean diameters of 83 and 86 respectively when unfertilized and fertilized. According to these results the eggs of *Arbacia punctulata* do not decrease in size as a result of fertilization under normal conditions but become larger instead. Loeb[4] found that the volume of the egg of *Strongylocentrotus* does not change appreciably as a result of fertilization. He says: "Ich habe eine Reihe von Messungen der Durchmessers des Cytoplasmas vor und nach der Befruchtung mit Hilfe des Zeichenapparates ausgefuhrt, wohin es sich herausstellte, dass das Volumen des Eicytoplasmas von *Strongylocentrotus* bei der

[1] Contribution from the Zoölogical Laboratory of the University of Michigan, No. 141.

[2] Calberla, Ernst, *Zeitschr. f. wiss. Zool.*, Vol. 30, 1877.

[3] McClendon, J. F., *Science*, N.S., Vol. 32, 1910.

[4] Loeb, J., *Arch. f. Entwicklungsmechanik*, Vol. 26, 1908.

Membranbildung keine mit dieser Methode wahrnehmbare Volumänderung erfährt. Das beweist mit Sicherheit, dass der Inhalt des Membranraums hauptsächlich Seewasser ist, das vom aussen in demselben diffundiert."

In some work that I have done[1] on the fertilization process in the brook lamprey, *Entosphenus wilderi* (Gage), I have obtained

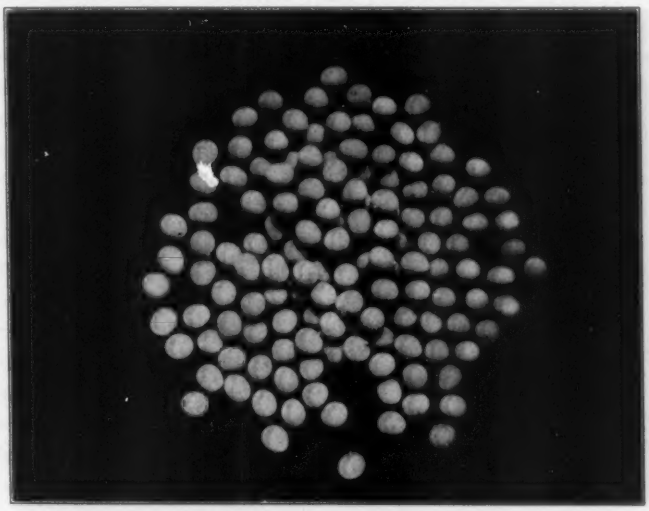

FIG. 1. Unfertilized eggs of *Entosphenus wilderi*.

results which indicate that the eggs in this form decrease considerably in size after fertilization.

Herfort[2] has given a careful description of the egg of *Petromyzon fluviatilis* and I have verified most of his observations on the egg of the American brook lamprey. Since there is no essential difference between the eggs of the two species, only a brief description is necessary here. Before fertilization the egg approaches very closely to an ellipsoid of revolution in shape although it is slightly more pointed at the animal pole (Fig. 1). It

[1] Presented to the Michigan Academy of Science at its nineteenth annual meeting in Ann Arbor, April 3, 1913. Fifteenth Report of the Michigan Academy of Science.

[2] Herfort, Karl, *Arch. f. mikr. Anat.*, Vol. 57, 1901.

possesses a distinct membrane which has been termed by Lubosch[1] and others the zona radiata but to which I shall apply the more general term vitelline membrane. In the ovarian egg of the larva this membrane consists of a single layer but after metamorphosis it becomes differentiated into two distinct layers which increase in thickness as the egg approaches maturity. The outer layer usually shows faint striations which have been supposed to represent canals. The two layers are very firmly united. Beneath the vitelline membrane there is a cortical alveolar layer which is covered on the outside by a very thin protoplasmic layer (vitelline membrane of Lubosch). This thin protoplasmic layer adheres very closely to the vitelline membrane and is continuous with the rest of the egg protoplasm through the walls of the alveoli. The alveolar layer seems to be similar to that in *Nereis* as described by F. R. Lillie.[2] The same condition has also been described by Reighard[3] in the egg of the wall-eyed pike. I have not been able to find any perivitelline space in the unfertilized egg.

Outside of the vitelline membrane there is a gelatinous substance which becomes adhesive upon the addition of water. This substance is supposed by Böhm[4] and others to be derived from the follicular epithelium of the ovarian egg. It is thickest at the vegetative pole and thins off gradually towards the animal pole. Here it is covered by a mucous mass which was first described by A. Müller[5] who called it the "Flocke." It is in the "Flocke" that most of the spermatozoa are found after the egg is fertilized. The vitelline membrane is slightly modified at the animal pole to form a region permeable to the spermatozoa but there appears to be no true micropyle present.

After fertilization visible changes almost immediately take place which result in the separation of the vitelline membrane from the egg and the formation of a large perivitelline space (Fig. 2). This separation is initiated a short distance from the middle of the animal pole as an indentation of the cortical layer, and a

[1] Lubosch, W., *Jen. Zeitschr. f. Naturwiss.*, Vol. 38, 1904.

[2] Lillie, F. R., *Jour. Morph.*, Vol. 22, 1911.

[3] Reighard, Jacob, Tenth Biennial Report of the State Board of Fish Commissioners (Michigan), 1893.

[4] Böhm, A. A., *Arch. f. Mikr. Anat.*, Vol. 32, 1888.

[5] Müller, A., Herrn Karl von Baer zur Feier des 50. Jahrestages, u.s.w., 1864.

circular perivitelline space is formed which extends around the pole in the form of a groove. This groove widens rapidly towards the animal pole until it forms a large space separating the egg at this pole from the vitelline membrane. At the same time a wave of contraction passes over the egg towards the vegetative pole and within a few minutes the whole egg is separated from the membrane. During the contractions of the egg, the long and short diameters vary considerably from time to time. I have

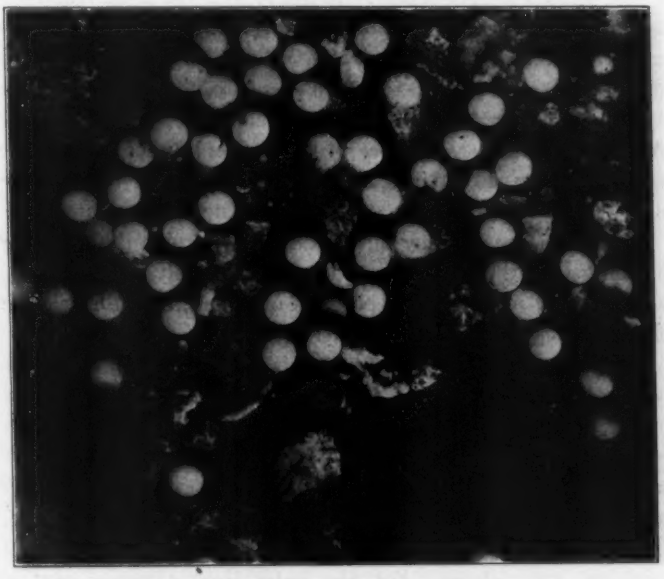

FIG. 2. Fertilized eggs of *Entosphenus wilderi*. ×6.

made accurate measurements at short intervals which show that the contractions are real and not merely apparent. They are very striking and remind one of peristaltic waves or ameboid movements.

The whole process during which the membrane separates from the egg lasts about five minutes and when the egg comes to rest again it has assumed a spherical shape instead of the previous ellipsoidal or ovoidal shape. During the process the vitelline membrane becomes greatly distended. When the membrane

first begins to separate from the egg, the walls of the alveoli of the cortical layer are drawn out into long slender threads which soon break, the outer part adhering to the vitelline membrane and the inner part fusing with the cytoplasm of the surface of the egg. The contents of the alveoli do not seem to diffuse through the vitelline membrane but remain in the perivitelline space. The thin layer of cytoplasm which adheres to the vitelline membrane seems to be similar to the same layer found in the fertilized egg of *Nereis*. In this form F. R. Lillie regards it as a plasma membrane "comparable in some respects to the fertilization membrane of the sea urchin" (loc. cit., page 364). This thin film does not seem to be a permanent structure in the lamprey egg and probably breaks up as the vitelline membrane distends. A part of it, at least, together with the cytoplasm of the alveolar walls seems to collect into little drops which float off into the fluid of the perivitelline space and eventually disintegrate.

Without going into any further detail concerning the structure of the egg and the changes it undergoes as a result of fertilization, we may now return to our problem of determining the relative size of the egg before and after fertilization.

The egg of the brook lamprey is noticeably smaller after fertilization than before. From careful measurements the volume of the egg before and after fertilization may be calculated with some degree of accuracy and the decrease in volume thus ascertained. In the case of an egg that is spherical in shape both before and after fertilization, as for instance the sea urchin egg, the diameter alone is the only dimension needed in order to calculate the volume. For the unfertilized lamprey eggs a different method must be employed. For purposes of calculation they were considered as perfect ellipsoids of revolution. This introduces an error but I am convinced it can not be very great, as the eggs are very nearly ellipsoidal in shape.

The unfertilized eggs were placed in a watch glass containing ordinary tap water at a temperature of about 19 degrees Centigrade and outlined by means of a camera lucida at a magnification of 80 diameters. These outlines were as nearly as possible optical sections through the plane of the major axis of the eggs and did not include the vitelline membrane. The eggs were then

fertilized and after about ten minutes they were outlined again in a similar manner through the planes of their diameters. The drawings thus obtained were then measured by means of a planimeter which recorded the area in square centimeters. From these areas and the long diameters in the case of the unfertilized eggs, and the areas and the diameters in case of the fertilized eggs, the volumes were calculated according to formulas as follows: The volume of an ellipsoid of revolution is equal to the constant .8488 times the square of the area of the plane of its major axis divided by the major axis; and the volume of a sphere is equal to the constant .8488 times the square of the area of its great circle divided by the diameter.[1] Formulas involving the use of planimeter areas and diameters were used because they seemed to introduce the least number of factors and thus eliminate chances of error. Since the same factors were used in each case the errors ought to average up about the same for both sets of drawings.

The volumes of 48 eggs at a magnification of 80 diameters were thus ascertained before and after fertilization. The volumes thus obtained were divided by the cube of 80 in order to get the actual volumes of the eggs. This gave as a result an average volume of .6017 cubic millimeter for the unfertilized eggs and an average volume of .5205 cubic millimeter for the fertilized eggs. This shows that the average decrease in size is .0812 cubic millimeter or 13.48 per cent. of the original volume.[2]

The above results are so striking that they have seemed worth recording at this time. Analogous conditions have been found by O. C. Glaser[3] in the egg of *Asterias forbesii*. Here the egg

[1] I am indebted to Professor Theodore R. Running for suggesting the use of these formulas.

[2] Professor Reighard has called my attention to the fact that since the unfertilized eggs are heavier than the water in which they are kept and rest on a plane surface, they may be slightly flattened by gravity. The fertilized eggs, on the other hand, which are immersed in a perivitelline fluid of greater density than water and rest on the curved vitelline membrane, are less likely to be flattened by gravity. For these reasons my measurements for the unfertilized eggs might be larger than those for the fertilized. I have examined unfertilized eggs that have been fixed in various fixing solutions but have found no evidence of flattening in these. There is no fresh material available at present. Since the eggs are small and were kept in water when they were outlined it does not seem probable that they would flatten to any appreciable extent.

[3] Glaser, O. C., *Science*, N.S., Vol. 38, 1913.

separates after fertilization from the inner surface of a thin preëxisting membrane and the change does not seem to be due to changes in the fertilization membrane but to changes in the surface film of the egg. The egg of *Asterias* after separating from the membrane was found to be smaller than before. In case of the lamprey egg also the initial change seems to be in the cortical layer of the egg and not in the membrane.

I have succeeded in inducing the egg of the lamprey to go through changes similar to those it exhibits after fertilization, by subjecting it to artificial stimuli, such as shaking, pricking with a needle, exposing to sudden changes of temperature, etc. The membrane separates in a similar manner f om the egg, a peristaltic wave passes over it and it becomes spherical in shape. This indicates that the stimulus that starts the egg on a career of development need not be of any specific kind. Some of the lamprey eggs that have thus been stimulated have segmented parthenogenetically. Bataillon[1] has succeeded in inducing parthenogenetic development of the egg of *Petromyzon planeri* by introducing them into a solution of cane sugar, and he has demonstrated that the segmentation is real and not merely a fragmentation of the egg.

Most of the material that the eggs lose as a result of fertilization is probably water, but it seems to be certain that other substances are also given off. Reighard (loc. cit., page 105) called the fluid contents of the alveoli of the cortical layer in the egg of the wall-eyed pike "cortical drops" and came to the conclusion that these drops consisted of an albuminous substance and that this substance is in solution in the perivitelline fluid. F. R. Lillie (loc. cit., page 365) thinks that the contents of the cortical alveoli in the egg of *Nereis* are unquestionably colloidal. Similar conclusions have been reached by others in other forms. In the lamprey egg after fertilization much of the substance that fills up the space between the egg and the membrane is water that has diffused through from the outside. Some of the substance, however, came from the cortical alveoli and is probably a colloid. F. R. Lillie[2] has found that the unfertilized ova of *Nereis* and *Arbacia* in sea water secrete

[1] Bataillon, E., *Arch. f. Entwicklungsmechanik*, Vol. 18, 1904.

[2] Lillie, F. R., *Science*, N.S., Vol. 38, 1913.

substances which cause agglutination of their respective spermatozoa. He has termed this substance "fertilizin" and assumes that "the union of this substance with the spermatozoa enters in some significant way into the process of development," and that it is located in the cortex of the egg. It may be that the "Flocke" described for the lamprey egg has some significance in this connection for it is in it that the spermatozoa seem to collect in great numbers after fertilization.

Evidences presented in this paper show clearly that the egg of the brook lamprey decreases considerably in size after fertilization. This is contrary to the results obtained by Calberla in the egg of *Petromyzon planeri*, by McClendon in *Arbacia punctulata*, and by Loeb in *Strongylocentrotus*. It harmonizes, however, with the results obtained by Glaser in *Asterias forbesii*. There is reason for believing that some of the substance given off by the egg after fertilization is colloidal in nature. Much of this substance comes from the cortical alveoli.

I desire to express my thanks to Professor Jacob Reighard for his criticism in connection with the preparation of this paper.

ANN ARBOR, MICH.,
November 17, 1913.